



FINAL REPORT

Test Facility Study No. 511871

IN VITRO SKIN CORROSION TEST WITH MLA-3202 USING A HUMAN SKIN MODEL

SPONSOR:

Chemtura Corporation
199 Benson Road
MIDDLEBURY, CT 06762
USA

TEST FACILITY:
WIL Research Europe B.V.
Hambakenwetering 7
5231 DD ‘s-Hertogenbosch
The Netherlands

27 May 2016

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2. STATEMENT OF GLP COMPLIANCE

WIL Research Europe B.V., 's-Hertogenbosch, The Netherlands

All phases of this study performed by the test facility were conducted in compliance with the following GLP regulations:

- OECD Principles of Good Laboratory Practice concerning Mutual Acceptance of Data in the Assessment of Chemicals, 26 November 1997 (C(97) 186 Final);
- EC Council Directive 2004 (2004/10/EC, February 11, 2004, Official Journal of February 20, 2004).

Except for the following:

- The quality environment in which the characterisation of the test item was performed was not known.

The data generated and reported are considered to be valid.

WIL Research Europe B.V.

Signature: 

Name: I.M.J. Eurlings, PhD.

Title: Study Director

Date: 27 May 2016

3. TEST FACILITY QUALITY ASSURANCE STATEMENT

WIL Research Europe B.V., 's-Hertogenbosch, The Netherlands.

Study title: *In vitro* skin corrosion test with MLA-3202 using a human skin model

This report was inspected by the WIL Research Europe Quality Assurance Unit (QAU) according to the Standard Operating Procedure(s).

The reported method and procedures were found to describe those used and the report reflects the raw data.

During the on-site process inspections, procedures applicable to this type of study were inspected.

The dates of Quality Assurance inspections are given below.

Project 511871

Type of Inspections	Phase/Process	Start Inspection date	End Inspection date	Reporting date
Study	Study Plan Report	29-Mar-2016 20-May-2016	29-Mar-2016 20-May-2016	29-Mar-2016 20-May-2016
Process	Test Substance Receipt Test Substance Handling	08-Feb-2016	29-Feb-2016	01-Mar-2016
	Genetic and In Vitro Toxicology Test Substance Handling Exposure Observations/Measurements Specimen Handling	22-Mar-2016	31-Mar-2016	04-Apr-2016

The facility inspection program is conducted in accordance with Standard Operating Procedure.

The review of the final report was completed on the date of signing this QA statement.

WIL Research Europe B.V.

Signature: 

Name:

C. Mitchell B.Sc., FRQA
Head of Quality Assurance

Date:



4. SUMMARY

This objective of this study was to evaluate the corrosivity potential of MLA-3202 using a human three dimensional epidermal model (EpiDerm (EPI-200)). The possible corrosive potential of MLA-3202 was tested through topical application for 3 minutes and 1 hour. This method was designed to be compatible with the following:

- OECD Guideline for the Testing of Chemicals No. 431 “*In Vitro* Skin Corrosion: Reconstructed Human Epidermis (RHE) Test Method” (adopted 28 July 2015)
- Method B.40 of Commission Regulation (EC) No. 440/2008

Batch RC-1045 of MLA-3202 was a clear amber-red liquid. MLA-3202 was applied undiluted (50 µl) directly on top of the skin tissue.

The positive control had a mean relative tissue viability of 7% after 3 minutes of exposure. The absolute mean OD₅₇₀ (optical density at 570 nm) of the negative control tissues was within the laboratory historical control data range. In the range of 20 - 100% viability, the Coefficient of Variation between tissue replicates was ≤ 5%, indicating that the test system functioned properly.

Skin corrosion is expressed as the remaining cell viability after exposure to the test item. The relative mean tissue viability obtained after 3-minute and 1-hour treatments with MLA-3202 compared to the negative control tissues was 67% and 86%, respectively. Because the mean relative tissue viability for MLA-3202 was not below 50% after the 3-minute treatment and not below 15% after the 1-hour treatment MLA-3202 is considered to be not corrosive.

Finally, it is concluded that this test is valid and that MLA-3202 is not corrosive in the *in vitro* skin corrosion test under the experimental conditions described in this report.

5. INTRODUCTION

5.1. Study schedule

Experimental starting date : 04 April 2016
Experimental completion date : 08 April 2016

5.1. Purpose

The objective of this study was to evaluate MLA-3202 for its ability to induce skin corrosion. For this purpose MLA-3202 was topically applied on a human three dimensional epidermal model.

Background of the test system

The test is based on the experience that corrosive chemicals show cytotoxic effects following short-term exposure to the EpiDerm skin model. Corrosive chemicals are able to penetrate the stratum corneum of the epidermis and are sufficiently cytotoxic to cause cell death in the underlying layers. Cytotoxicity is expressed as the reduction of mitochondrial dehydrogenase activity measured by formazan production from 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) at the end of the treatment.

The test is designed to predict and classify the skin corrosion potential of a test item by assessment of its effect on a three-dimensional human epidermis model (1-4).

The test consists of topical application of MLA-3202 on the skin tissue for 3-minute and 1-hour. After exposure the skin tissue is thoroughly rinsed to remove the test item followed by immediate determination of the cytotoxic (corrosive) effect.

5.2. Guidelines

The study procedures described in this report are in compliance with the following guidelines:

- Organisation for Economic Co-operation and Development (OECD), OECD Guidelines for Testing of Chemicals, Guideline no. 431: *In Vitro Skin Corrosion: reconstructed human epidermis (RHE)* test method (adopted 28 July 2015).
- European Community (EC). Commission regulation (EC) No. 440/2008, Part B: Methods for the Determination of Toxicity and other health effects, Guideline B.40 BIS: "*In Vitro Skin Corrosion: Human Skin Model Test*". Official Journal of the European Union No. L142, 31 May 2008.

5.3. Retention of records and materials

Records and material pertaining to the study, which include study plan and amendments, raw data and the final report will be retained in the archives of the test facility for a minimum of 5 years after the finalization of the report. After this period, the sponsor will be contacted to determine how the records and materials should be handled. The test facility will retain information concerning decisions made.

A sample of the test item will be retained until expiry date or applicable retest date. After this period the sample(s) will be destroyed.

5.4. Responsible personnel

5.4.1. Test facility

Study Director I.M.J. Eurlings, PhD.

5.4.2. Sponsor Representative

Study Monitor Audrey Batoon, Ph.D.

6. MATERIALS AND METHODS

6.1. Test item

6.1.1. Test item information

Test item	207258/A
Identification	MLA-3202
Appearance	Clear amber-red liquid
Batch	RC-1045
Purity/Composition	UVCB
Test item storage	At room temperature
Stable under storage conditions until	17 February 2019 (expiry date)

6.1.2. Study specific test item information

Purity/composition correction factor	No correction factor required
Test item handling	No specific handling conditions required
Chemical name (IUPAC), synonym or trade name	Amides, tallow, N,N-bis(2-hydroxypropyl)
CAS Number	1454803-04-3

6.2. Reference items

Negative control:

Milli-Q water (Millipore Corp., Bedford, Mass., USA).

Positive control:

Potassium hydroxide (KOH; Merck, Darmstadt, Germany), an 8.0 normal solution was prepared.

6.3. Test item preparation

No correction was made for the purity/composition of the test item.

The liquid test item was applied undiluted (50 µl) directly on top of the tissue. MLA-3202 was spread to match the size of the tissue.

6.4. Test system

Test system

EpiDerm Skin Model (EPI-200, Lot no.: 23697 kit U and T, [APPENDIX 4](#)).

The model consists of normal, human-derived epidermal keratinocytes which have been cultured to form a multi-layered, highly differentiated model of the human epidermis. It consists of organized basal, spinous and granular layers, and a multi-layered stratum corneum containing intercellular lamellar lipid layers arranged in patterns analogous to those found *in vivo*. The EpiDerm tissues (surface 0.6 cm²) were cultured on polycarbonate membranes of 10 mm cell culture inserts.

Rationale

Recommended test system in international guidelines (OECD and EC).

Source

MatTek Corporation, Ashland MA, U.S.A.

6.5. Cell culture

Tissues

On the day of receipt the tissues were kept on agarose and stored in the refrigerator. On the next day, at least one hour before starting the assay the tissues were transferred to 6-well plates with 0.9 ml DMEM medium.

DMEM (Dulbecco's Modified Eagle's Medium)

Supplemented DMEM medium, serum-free supplied by MatTek Corporation.

MTT medium

MTT concentrate (5 mg/ml) diluted (1:5) with MTT diluent (supplemented DMEM). Both supplied by MatTek Corporation.

Environmental conditions

All incubations, with the exception of the test item incubation of 3 minutes at room temperature, were carried out in a controlled environment, in which optimal conditions were a humid atmosphere of 80 - 100% (actual range 69 - 90%), containing $5.0 \pm 0.5\%$ CO₂ in air in the dark at $37.0 \pm 1.0^\circ\text{C}$ (actual range 36.2 - 36.4°C). Temperature and humidity were continuously monitored throughout the experiment. The CO₂ percentage was monitored once on each working day. Temporary deviations from the temperature, humidity and CO₂ percentage may occur due to opening and closing of the incubator door. Based on laboratory historical data these deviations are considered not to affect the study integrity.

6.6. Study design

6.6.1. Test for the interference of the test item with the MTT endpoint

A test item may interfere with the MTT endpoint if it is coloured and/or it is able to directly reduce MTT. The cell viability measurement is affected only if the test item is present on the tissues when the MTT viability test is performed.

6.6.2. Test for colour interference by the test item

MLA-3202 was checked for possible colour interference before the study was started. Some non-coloured test items may change into coloured items in aqueous conditions and thus stain the skin tissues during the 1-hour exposure. To assess the colour interference, 50 µl of MLA-3202 or 50 µl Milli-Q water as a negative control were added to 0.3 ml Milli-Q water. The mixture was incubated for approximately 1 hour at $37.0 \pm 1.0^\circ\text{C}$ in the dark. At the end of the exposure time the mixture was shaken and it was checked if a blue / purple colour change was observed.

In case the test item induces colour interference in aqueous conditions, in addition to the normal procedure, two tissues must be treated with test item for 3 minutes and two tissues for 1-hour. Instead of MTT solution these tissues will be incubated with DMEM medium.

6.6.3. Test for reduction of MTT by the test item

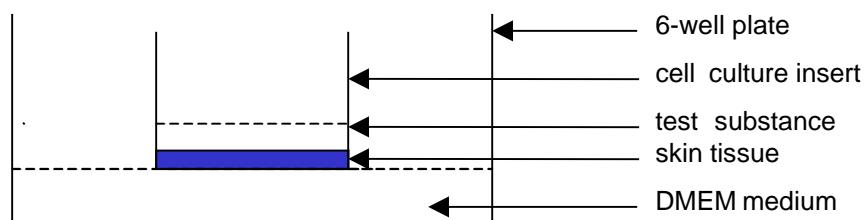
MLA-3202 was checked for possible direct MTT reduction before the study was started. To assess the ability of the test item to reduce MTT, 50 µl of MLA-3202 was added to 1 ml MTT (Sigma, Zwijndrecht, The Netherlands) solution (1 mg/ml) in phosphate buffered saline. The mixture was incubated for approximately 1 hour at $37.0 \pm 1.0^\circ\text{C}$. A negative control, sterile Milli-Q water was tested concurrently. At the end of the exposure time it was checked if a blue / purple colour change or a blue / purple precipitate was observed.

In case the test item reacts with the MTT medium in addition to the normal 1-hour procedure, two freeze-killed tissues treated with test item and two freeze-killed non treated tissues must be used for the cytotoxicity evaluation with MTT.

6.6.4. Application/Treatment of the test item

The skin tissues were kept in the refrigerator the day they were received. The next day, at least 1 hour before the assay was started the tissues were transferred to 6-well plates containing 0.9 ml DMEM medium per well. The level of the DMEM medium was just beneath the tissue (see fig 1). The plates were incubated for approximately 2 hours at $37.0 \pm 1.0^{\circ}\text{C}$. The medium was replaced with fresh DMEM medium just before MLA-3202 was applied. The test was performed on a total of 4 tissues per test item together with a negative control and positive control. Two tissues were used for a 3-minute exposure to MLA-3202 and two for a 1-hour exposure. Fifty μl of the undiluted test item was added into the 6-well plates on top of the skin tissues. The remaining tissues were treated with 50 μl Milli-Q water (negative control) and with 50 μl 8N KOH (positive control), respectively. After the exposure period, the tissues were washed twenty times with phosphate buffered saline (Invitrogen Corporation, Breda, The Netherlands) to remove residual test item. Rinsed tissues were kept in 24 well plates on 300 μl DMEM medium until 6 tissues (= one application time) were dosed and rinsed.

Figure 1 A diagram of the application



6.6.5. Cell viability measurement

The DMEM medium was replaced by 300 μl MTT-medium and tissues were incubated for 3 hours at 37°C in air containing 5% CO_2 . After incubation the tissues were washed with PBS and formazan was extracted with 2 ml isopropanol (MatTek corporation) over night at room temperature. The amount of extracted formazan was determined spectrophotometrically at 570 nm in triplicate with the TECAN Infinite® M200 Pro Plate Reader.

Cell viability was calculated for each tissue as percentage of the mean of the negative control tissues. Skin corrosion potential of the test item was classified according to remaining cell viability following exposure of the test item with either of the two exposure times.

6.7. Interpretation

6.7.1. Acceptability of the assay

The *in vitro* skin corrosion test is considered acceptable if it meets the following criteria:

- a) The absolute mean OD₅₇₀ of the two tissues of the negative control should reasonably be within the laboratory historical control data range.
- b) The mean relative tissue viability following 3-minute exposure to the positive control should be $\leq 30\%$.
- c) In the range 20 - 100% viability, the Coefficient of Variation (CV) between tissue replicates should be $\leq 30\%$.

6.7.2. Data evaluation and statistical procedures

A test item is considered corrosive in the skin corrosion test if:

- a) The relative mean tissue viability obtained after 3-minute treatment compared to the negative control tissues is decreased below 50%.
- b) In addition, a test item considered non-corrosive (viability $\geq 50\%$) after the 3-minute treatment is considered corrosive if the relative tissue viability after 1-hour treatment with the test item is

decreased below 15%.

A test item is considered non corrosive in the *in vitro* skin corrosion test if:

- a) The relative mean tissue viability obtained after the 3-minute treatment compared to the negative control tissues is not decreased below 50%.
- b) In addition, the relative tissue viability after the 1-hour treatment is not decreased below 15%.
Table 1 presents the data interpretation and optional sub-categorisation in case a test item will be corrosive.

Table 1 Data interpretation and sub-categorisation of test items

Viability measured after 3-minutes and 1 hour	Prediction to be considered
< 50% after 3 minute exposure	Corrosive: sub-category 1A (optional)
≥ 50% after 3 minute exposure AND < 15% after 1 hour exposure	Corrosive: sub-category 1B and 1C (optional)
≥ 50% after 3 minute exposure AND ≥ 15% after 1 hour exposure	Non-corrosive

6.8. List of deviations

6.8.1. List of study plan deviations

There were no deviations from the study plan.

6.8.2. List of standard operating procedures deviations

Any deviations from standard operating procedures were evaluated and filed in the study file. There were no deviations from standard operating procedures that affected the integrity of the study.

7. ELECTRONIC SYSTEMS FOR DATA ACQUISITION

The following electronic systems were used for data acquisition:

- REES Centron Environmental Monitoring system version SQL 2.0 (REES Scientific, Trenton, NJ, USA): Temperature and humidity.
- Magellan Tracker 7.0 (TECAN, Austria) for optical density measurement.

8. RESULTS

MLA-3202 was checked for colour interference in aqueous conditions and possible direct MTT reduction by adding the test item to MTT medium. Because the solutions did not turn blue / purple nor a blue / purple precipitate was observed it was concluded that MLA-3202 did not interfere with the MTT endpoint.

The mean absorption at 570 nm measured after treatment with MLA-3202 and controls are presented in [APPENDIX 1, Table 2](#). The individual OD₅₇₀ measurements are presented in [APPENDIX 2](#).

Table 3 shows the mean tissue viability obtained after 3-minute and 1-hour treatments with MLA-3202 compared to the negative control tissues. Skin corrosion is expressed as the remaining cell viability after exposure to the test item. The relative mean tissue viability obtained after the 3-minute and 1-hour treatments with MLA-3202 compared to the negative control tissues was 67% and 86%

respectively. Because the mean relative tissue viability for MLA-3202 was not below 50% after 3 minutes treatment and not below 15% after 1 hour treatment MLA-3202 is considered to be not corrosive.

The absolute mean OD₅₇₀ (optical density at 570 nm) of the negative control tissues was within the laboratory historical control data range (See [APPENDIX 3](#)). The mean relative tissue viability following 3-minute exposure to the positive control was 7%.

In the range of 20 - 100% viability the Coefficient of Variation between tissue replicates was ≤ 5%, indicating that the test system functioned properly ([APPENDIX 1, Table 4](#)).

9. CONCLUSION

Finally, it is concluded that this test is valid and that MLA-3202 is not corrosive in the *in vitro* skin corrosion test under the experimental conditions described in this report.

10. REFERENCES

1. Kibilus, J., Cannon, C., Neal, P., Senott, H., Klausner, M.(1996). Response of the EpiDerm skin model to topically applied irritants and allergens. *In vitro toxicology* 9: 157-166.
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4. Barratt, M.D., Brantom, P.G., Fentem, J. H., Gerner, I., Walker A.P., Worth, A.P. (1998). The ECVAM international validation study on *in vitro* tests for skin corrosivity. 1. Selection and distribution of the test chemicals. *Toxicology in vitro* 12: 471 - 482.
5. Fentem, J. H., Archer, G. E. B., Balls, M., Botham, P. A., Curren, R. D., Earl, L. K., Esdaile, D. J., Holzhütter, H., Liebsch, M. (1998). The ECVAM international validation study on *in vitro* tests for skin corrosivity. 2. Results and evaluation by the management team. *Toxicology in vitro* 12: 483 - 524.
6. Ponec, M., Boelsma, E., Weerheim, A., Mulder, A., Bouwstra, J., Mommaas, M. (2000). Lipid and ultrastructural characterization of reconstructed skin models. *International Journal of Pharmaceutics* 203: 211 - 225.

APPENDIX 1 TABLES**Table 2 Mean absorption in the *in vitro* skin corrosion test with MLA-3202**

	3-minute application				1-hour application			
	A (OD ₅₇₀)	B (OD ₅₇₀)	Mean (OD ₅₇₀)	SD	A (OD ₅₇₀)	B (OD ₅₇₀)	Mean (OD ₅₇₀)	SD
Negative control	2.144	2.239	2.192	± 0.067	2.033	2.081	2.057	± 0.034
MLA-3202	1.439	1.496	1.467	± 0.040	1.764	1.756	1.760	± 0.006
Positive control	0.146	0.152	0.149	± 0.004	0.141	0.218	0.179	± 0.055

SD = Standard deviation

Duplicate exposures are indicated by A and B.

In this table the values are corrected for background absorption (0.0405). Isopropanol was used to measure the background absorption.

Table 3 Mean tissue viability in the *in vitro* skin corrosion test with MLA-3202

	3-minute application viability (percentage of control)	1-hour application viability (percentage of control)
Negative control	100	100
MLA-3202	67	86
Positive control	7	9

Table 4 Coefficient of Variation between tissue replicates

	3 minute	1 hour
Negative control	4.3	2.3
MLA-3202	3.8	0.5
Positive control	3.9	35.5

$$CV (\%) = 100 - [(\text{lowest OD}570 / \text{highest OD}570) \times 100\%]$$

APPENDIX 2 INDIVIDUAL OD MEASUREMENTS AT 570 NM

	3-minute application (OD_{570})		1-hour application (OD_{570})	
	A	B	A	B
Negative control				
OD_{570} measurement 1	2.1973	2.3017	2.1016	2.1050
OD_{570} measurement 2	2.1816	2.2773	2.0743	2.1339
OD_{570} measurement 3	2.1752	2.2608	2.0458	2.1257
MLA-3202				
OD_{570} measurement 1	1.4956	1.5285	1.8073	1.8057
OD_{570} measurement 2	1.4680	1.5558	1.7824	1.7708
OD_{570} measurement 3	1.4756	1.5246	1.8248	1.8121
Positive control				
OD_{570} measurement 1	0.1862	0.1939	0.1827	0.2583
OD_{570} measurement 2	0.1874	0.1910	0.1816	0.2639
OD_{570} measurement 3	0.1870	0.1934	0.1792	0.2537

OD = Optical density

Duplicate exposures are indicated by A and B.

APPENDIX 3 HISTORICAL CONTROL DATA FOR IN VITRO SKIN CORROSION STUDIES

	Negative control		Positive control		Positive control	
	3-minute treatment (OD ₅₇₀)	1-hour treatment (OD ₅₇₀)	3-minute treatment (OD ₅₇₀)	1-hour treatment (OD ₅₇₀)	3-minute treatment (% viability)	1-hour treatment (% viability)
Range	1.324 – 2.615	1.361 – 2.352	0.172 – 0.56	0.057 – 0.277	6 – 22	3 – 12
Mean	1.86	1.86	0.18	0.13	10.67	7.17
SD	0.24	0.22	0.10	0.05	3.9	2.36
n	65	67	64	61	30	30

SD = Standard deviation

n = Number of observations

The above mentioned historical control data range of the controls were obtained by collecting all data over the period of December 2012 to December 2015.

APPENDIX 4 CERTIFICATE OF ANALYSIS EPIDERM SKIN MODEL

Certificate of Analysis**Product: EpiDerm™ Reconstructed Human Epidermis**

Lot Number: 23697

Part #: EPI-200, EPI-212

Description: Reconstructed human epidermis tissue containing normal human keratinocytes. This product is for research use only. Not for use in animals, humans or diagnostic purposes.

I. Cell source

All cells used to produce EpiDerm™ are purchased or derived from tissue obtained by MatTek Corporation from accredited institutions. In all cases, consent was obtained by these institutions from the donor or the donor's legal next of kin, for use of the tissues or derivatives of the tissue for research purposes.

Keratinocyte Strain: 00267

II. Analysis for potential biological contaminants

The cells used to produce EpiDerm™ tissue are screened for potential biological contaminants. Tests for each potential biological contaminant listed below were performed according to the test method given. Results of "Not detected" indicate that testing for the potential biological contaminant was not observed as determined by the stated test method.

Keratinocytes:

HIV-1 virus - Oligonucleotide-directed amplification	Not detected
Hepatitis B virus - Oligonucleotide- directed amplification	Not detected
Hepatitis C virus - Oligonucleotide- directed amplification	Not detected
Bacteria, yeast, and other fungi - long term antibiotic, antimycotic free culture	Not detected

III. Analysis for tissue functionality and quality

Test	Specification	Acceptance criteria	Result and QA Statement	
Tissue viability	MTT QC assay, 4 hours, n=3	OD (540-570 nm) <1.0-3.0>	1.712 ± 0.026	Pass
Barrier function	ET-50 assay, 100 µl 1% Triton X-100, 4 time-points, n=3, MTT assay	ET-50 <4.77-8.72 hrs>	7.25 hrs	Pass
Sterility	Long term antibiotic and antimycotic free culture	No contamination	Sterile	Pass

Tissue viability and the barrier function test are within the acceptable ranges and indicate appropriate formation of the epidermal barrier, the presence of a functional stratum corneum, a viable basal cell layer, and intermediate spinous and granular layers. Results obtained with this lot conform to the requirements of the OECD TG 431 and 439.

Initials:

TYK
4/6/16

Date:

Paul Kearney
Paul Kearney
Quality Assurance Manager

April 6, 2016

Date

CAUTION: Whereas all information herein is believed to be correct, no absolute guarantee that human derived material is non-infectious can be made or is implied by this certificate of analysis. All tissues should be treated as potential pathogens. The use of protective clothing and eyewear and appropriate disposal procedures are strongly recommended.

MatTek Corporation
200 Homer Avenue, Ashland, MA - USA
+1-508-881-6771

www.mattek.com
information@mattek.com

APPENDIX 5 CERTIFICATE OF ANALYSIS



Chempura Corporation
12 Spencer St
Naugatuck, CT 06770

Analytical Services
www.chemtura.com

Certificate of Purity

Customer: Support for Toxicology Studies

Test Substance Name: MLA3202; Amides, tallow, N,N-bis(2-hydroxypropyl)

Physical Appearance: Liquid

CAS No.: 1454803-04-3

Ref. or Lot Number: RC-1045

Date of Analysis: revised March 18, 2016 (original issue March 7, 2016)

Percent Composition	Monoisotopic Mass (daltons)	Formula	Structure/ Identity
33.1	397.4	C ₂₄ H ₄₇ NO ₃	C18:1 (oleic) tallow amides, N,N-bis(2-hydroxypropyl)
22.9	371.3	C ₂₂ H ₄₅ NO ₃	C16:0 (palmitic) tallow amides, N,N-bis(2-hydroxypropyl)
13.6	395.4	C ₂₄ H ₄₅ NO ₃	C18:2 (linoleic) tallow amides, N,N-bis(2-hydroxypropyl)
11.0	399.4	C ₂₄ H ₄₉ NO ₃	C18:0 (stearic) tallow amides, N,N-bis(2-hydroxypropyl)
6.0	369.3	C ₂₂ H ₄₃ NO ₃	C16:1 (palmitoleic) tallow amides, N,N-bis(2-hydroxypropyl)
3.2	419.3	C ₂₆ H ₄₅ NO ₃	C20:4 (eicosatetraenoic) tallow amides, N,N-bis (2-hydroxypropyl)
2.0	393.3	C ₂₄ H ₄₃ NO ₃	C18:3 (linolenic) tallow amides, N,N-bis(2-hydroxypropyl)
1.5	282.3	C ₁₈ H ₃₄ O ₂	C18:1 (oleic) acid
1.1	421.4	C ₂₆ H ₄₇ NO ₃	C20:3 (eicosatrienoic) tallow amides, N,N-bis (2-hydroxypropyl)
5.6			Sum of residual components (< 1% each)
100.0			Total

Blake Lewis 3/7/16
 Blake Lewis
 Analytical REACH Scientist, Analytical Services
Colin Moore 3/7/16
 Albert J. Nitowski
 Sr. Technology Manager
 Analytical and Lab Support Services